DR.
10-28-98

CANINE MILK SUBSTITUTE

BACKGROUND OF THE INVENTION

This invention relates to a canine milk substitute, or bitch milk replacer, which substantially supplies the nutritional requirements of nursing puppies.

In recent years, the nutritional requirements of growing puppies as well as adult dogs have been studied; however, little information has been acquired on the composition of canine milk and how it meets the nutritional requirements of nursing puppies. It is generally accepted that milk from the lactating mother provides optimal nutrition to the suckling puppy. Accordingly, milk replacers currently in use have been formulated with the intent of matching the nutrient composition of bitch milk. However, most of the commercially known formulations are based on studies conducted with regard only to crude protein, fat and mineral concentrations in the milk of lactating mothers. See Lonnerdal et al., "Developmental Changes in the Composition of Beagle Dog Milk", *Am. J. Vet. Res.*, Vol. 42:662-666 (1981) and Oftedal, "Lactation in the Dog: Milk Composition and Intake by Puppies", *J. Nutr.*, 114:803-812 (1984). Little study has been devoted to important information such as fatty acid and amino acid profiles in bitch milk and other factors which are believed to have an effect on the nutrient profiles of bitch milk including breed and stage of lactation.

Accordingly, as currently available commercial bitch milk replacers have been formulated based on limited research data, they do not necessarily provide adequate nutrition to the nursing puppy to ensure proper development and growth. Therefore, there is still a need in the art for a canine milk replacer which is based more closely on the actual concentrations of essential nutrients in bitch milk and which substantially supplies the nutritional needs of nursing puppies.

SUMMARY OF THE INVENTION

The present invention meets that need by providing a canine milk substitute, or milk replacer, containing amounts of protein, fat and carbohydrates which closely matches the concentrations of those components in bitch milk. In addition, the milk replacer of the present

invention provides improved fatty acid and amino acid profiles over currently available bitch milk replacers, and has been found to enhance growth in nursing puppies as compared to currently available commercial products.

In accordance with one aspect of the present invention, an artificially produced canine milk substitute composition is provided which comprises, on a dry matter basis (DMB), from about 35 to 45% by weight protein, from about 25 to 35% by weight fat, and from about 10 to 25% by weight carbohydrates. In a preferred embodiment of the invention, the composition comprises about 38% protein, about 28% fat, and about 19% carbohydrates.

The protein source preferably comprises casein and whey in a weight ratio of about 70:30. The source of fat is preferably selected from the group consisting of corn oil, canola oil, butter oil, arachidonic acid, docosahexaenoic acid and blends thereof.

The composition of the present invention also preferably contains fatty acids comprising, as a percentage of total fatty acids on a dry matter basis, from about 15 to 19% palmitic acid, from about 5 to 9% stearic acid, from about 34 to 38% oleic acid, from about 17 to 21% linoleic acid, from about 1 to 4% α-linolenic acid, from about 0.5 to 2% arachidonic acid, from about 0.2 to 1.0% docosahexaenoic acid (DHA), from about 2 to 5% Omega 3 fatty acids, from about 18 to 22% Omega 6 fatty acids, and from about 1 to 4% trans fatty acids. The composition preferably contains 27 to 37% by weight fatty acids on a dry matter basis.

The composition also contains essential amino acids comprising, as a percentage of total essential amino acids on a dry matter basis, from about 6 to 10% arginine, 4 to 8% histidine, 8 to 12% isoleucine, 16 to 20% leucine, from about 13 to 17% lysine, from about 2 to 7% methionine, from about 6 to 10% phenylalanine, from about 8 to 12% threonine, from about 1 to 4% tryptophan, from about 9 to 13% valine, from about 2 to 5% cystine, and from about 2 to 6% tyrosine. The composition preferably contains from about 15 to 25% by weight essential amino acids on a dry matter basis.

The composition also preferably contains, on a dry matter basis, from about 4 to 8% by weight lactose and from about 0.50% by weight fructooligosaccharide. The composition may also include mixtures of vitamins and minerals.

When the composition of the present invention is fed to puppies in a quantity and frequency appropriate for their nutritional needs, it has been found that the puppies exhibit exceptional growth performance which is superior to other currently available canine milk replacers and which is very similar in pattern to maternally reared puppies.

Accordingly, it is a feature of the present invention to provide a canine milk substitute that duplicates canine milk more closely than currently available products and which substantially supplies the nutrient requirements of nursing puppies. Other features and advantages of the invention will be apparent from the following description, the accompanying drawings, and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a bar graph showing percentages of protein, fat and carbohydrates in the composition of the present invention compared with those contained in commercially available bitch milk replacers and bitch milk;

Fig. 2 is a bar graph showing the fatty acid profile of the composition of the present invention compared with the profiles of commercially available bitch milk replacers and bitch milk; and

Fig. 3 is a bar graph showing the amino acid profile of the composition of the present invention compared with the profiles of commercially available bitch milk replacers and bitch milk.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The milk substitute composition of the present invention provides an improvement over currently available milk substitutes in that it more closely matches the nutrient profile of bitch milk. The composition of the present invention is higher in protein and lower in fat content that currently commercially available milk replacers. For example, the composition of the present invention preferably comprises at least 38% by weight protein in comparison to currently available milk replacers which comprise only about 29 to 34% protein. In addition, the composition of the

present invention comprises only about 28% by weight fat in comparison to currently available milk replacers which comprise from about 33 to 42% fat.

The present invention also provides amino acid profiles and fatty acid profiles which are closer to that of bitch milk than commercially available products. When the milk replacer composition of the present invention is fed to puppies on a daily basis, it has been found to provide greater daily weight gain and improved growth performance than commercially available milk replacer products.

The composition is preferably provided in powder form, but may be provided in any suitable form (e.g., liquid) as long as it contains the preferred concentrations of protein, fat, and carbohydrates on a dry matter basis.

A study of bitch milk was undertaken to determine the concentration of true protein, total fat, total carbohydrates, the casein to whey ratio, the amino acid profile, and the fatty acid profile. The testing procedures and results are described below.

Milk samples (approximately 10 mL) were collected from a total of 10 beagle dogs on days 1, 3, 7, 14, 21, 28, 35 and 42 of lactation and stored frozen until subjected to laboratory analysis. All bitches were maintained on the same diet. Bitch and individual puppy body weights were recorded on the days of milk collection.

Nitrogen analysis--Total nitrogen, NPN, and true protein nitrogen were anlyzed by micro-Kjeldahl.

Casein:whey ratio--The casein to whey ratio was determined by gel electrophoresis following the determination of the optimal separation conditions for these two class of proteins.

Amino Acids--Amino acid profiles were determined for milk and for the whey and casein proteins.

Fatty Acids--Fatty acids profiles were analyzed by gas chromatography.

Lactose--Lactose was determined by the lactase assay and total lipids via the Folch procedure.

Minerals--Mineral composition was determined by atomic absorption spectrometry.

Milk proteins--Milk protein composition was determined by gel electrophoresis and by FPLC using gel filtration and ion-exchange chromatography. Isolated proteins were identified by N-terminal sequencing.

Results

Protein concentration was very high in day 1 samples (144 ± 60 g/L), then decreased through day 21 (68 ± 13 g/L); a modest increase was then observed. There was no change in the concentration of NPN (approximately 6-10% of total N). The casein/whey ratio was about 70/30 and stayed constant throughout lactation. Lactose concentration increased during early lactation: day 1 samples contained 17 ± 4 g/L, while day 7 to day 42 samples contained 34-40 g/L. Lipid concentration was high in early lactation(approximately 13%) and then decreased slightly (approximately 11%). Citrate concentration increased from day 1 to day 7 (4.8 ± 1.2 to 6.6 ± 1.0 mM), then gradually decreased by day 42 (3.9 ± 1.2 mM).

Although data was developed through the duration of lactation, only the data for day 7 of lactation are described below. Because milk composition changes throughout the course of lactation and the nutrient needs of puppies are most critical during the first two weeks of life, it was determined that this early milk composition was the most appropriate data upon which to base a single milk replacer formulation. All of the following data are presented on a dry matter basis. The percentages in the essential amino acid and fatty acid profiles are expressed as a percentage of total essential amino acids and total fatty acids, respectively.

Composition	
Protein	40.40%
Fat	31.8%*
Carbohydrate	18.5%
Casein/Whey Ratio	70:30

*This value was obtained from Lonnerdal et al., "Developmental Changes in the Composition of Beagle Dog Milk", *Am. J. Vet. Res.*, Vol. 42:662-666 (1981) and Oftedal, "Lactation in the Dog: Milk Composition and Intake by Puppies", *J. Nutr.*, 114:803-812 (1984).

Essential Amino Acid Profile (%)	
Arginine	9.70
Histidine	6.37
Isoleucine	8.94
Leucine	22.14
Lysine	8.92
Methionine	5.40
Cystine	3.84
Phenylalanine	7.83
Tyrosine	5.61
Threonine	8.25
Tryptophan	1.06
Valine	11.9

Fatty Acid Profile (%)	
Palmitic	24.30
Stearic	2.90
Oleic	36.5
Linoleic	14.5
Arachidonic	1.22
Docosahexaenoic	0.70
Trans Fatty acids	3.40

Based on this data, the bitch milk replacer of the present invention was formulated and preferably contains about 38% protein, about 28% fat, and about 19% carbohydrates. The casein and whey in the composition have a weight ratio of about 70:30.

The source of fat in the milk replacer composition preferably comprises a blend of corn oil, canola oil, butter oil, arachidonic acid, and docosahexaenoic acid. This blend of fats is believed to provide a fatty acid profile which is close to that of bitch milk and comprises from

about 15 to 19% palmitic acid, from about 5 to 9% stearic acid, from about 34 to 38% oleic acid, from about 17 to 21% linoleic acid, from about 1 to 4% α-linolenic acid, from about 0.5 to 2% arachidonic acid, from about 0.2 to 1.0% docosahexaenoic acid (DHA), from about 2 to 5% Omega 3 fatty acids, from about 18 to 22% Omega 6 fatty acids, and from about 1 to 4% trans fatty acids (expressed as a percentage of total fatty acids on a dry matter basis). The fatty acids preferably comprise about 25% to 35%, and most preferably, about 28% of the total composition on a dry matter basis.

The composition also contains amounts of essential amino acids which exhibit a profile similar to that of actual bitch milk. Expressed as a percentage of total essential amino acids on a dry matter basis, the amino acids preferably comprise from about 6 to 10% arginine, 4 to 8% histidine, 8 to 12% isoleucine, 16 to 20% leucine, from about 13 to 17% lysine, from about 2 to 7% methionine, from about 6 to 10% phenylalanine, from about 8 to 12% threonine, from about 1 to 4% tryptophan, from about 9 to 13% valine, from about 2 to 5% cystine, and from about 2 to 6% tyrosine. The essential amino acids preferably comprise about 15% to 25%, and most preferably, about 20% of the total composition on a dry matter basis.

The composition also preferably contains, on a dry matter basis, from about 4-8% by weight lactose and from about 0.50% by weight fructooligosaccharide. Fructooligosaccharide (FOS) is preferably included in the formulation because studies have shown FOS to be beneficial to the intestinal health of many animals. FOS may be metabolized by beneficial intestinal bacterial species, such as Bifidobacterium. However, harmful intestinal bacteria, such as Salmonella, E. coli and Clostridia are unable to process FOS. Therefore, FOS appear to promote a healthy intestinal environment in animals.

The composition may also contain vitamins and minerals including, but not limited to Vitamin A acetate, cholecalciferol, d, l-alpha tocopheryl acetate, cyanocobalamin, riboflavin, niacinaminde, d-calcium pantothenate, folic acid, thiamin mononitrate, pyridoxine hydrochloride, biotin, inositol, ascorbic acid, dextrose, tricalcium phosphate, potassium chloride, potassium citrate, magnesium sulfate, monosodium phosphate, zinc sulfate, copper sulfate, manganese sulfate, sodium selenite, potassium iodide, cobalt sulfate, and ferric methionine.

In order that the invention may be more readily understood, reference is made to the following example which is intended to illustrate the invention, but not limit the scope thereof.

Example 1

A milk substitute was prepared in accordance with the present invention by combining the following ingredients:

Formula A

Ingredient	Percentage
Water	80.0
Na/Ca Caseinate	5.233
Whey Protein Concentrate	3.491
Maltodextrin	2.646
Butter Oil	2.412
Canola Oil	1.764
Mineral Premix[1]	1.147
Lactose	1.134
Corn Oil	.869
Dried Egg Yolk	.506
Emulsifier	.200
Vitamin Premix[2]	.128
Arachidonic Acid Supplement	.100
Fructooligosaccharide	.100
L-Histidine HCl	.090
L-Arginine	.060
Choline Chloride	.055
DHA Supplement	.040
Ascorbic Acid	.025

[1]The "Mineral Premix" contained one or more of the following: tricalcium phosphate, potassium chloride, potassium citrate, magnesium sulfate, monosodium phosphate, zinc sulfate, copper sulfate, manganese sulfate, sodium selenite, potassium iodide, cobalt sulfate, and ferric methionine.

[2]The "Vitamin Premix" contained one or more of the following: Vitamin A acetate, cholecalciferol, d,l-alpha tocopheryl acetate, cyanocobalamin, riboflavin, niacinaminde, d-calcium pantothenate, folic acid, thiamin mononitrate, pyridoxine hydrochloride, biotin, inositol, and ascorbic acid.

To compare the formulation of the present invention with currently available products, an analysis of several commercially available bitch milk replacer formulas was conducted. The test formulas were JustBorn™ (JBD), available from Farnam Pet Products, Esbilac® Milk Replacer for Puppies (ESB), available from Pet-Ag, Inc., and Nurturall Puppy Balanced Milk Replacer (Nurtal), available from Veterinary Products Laboratory. Table 1 shows the protein, fat and carbohydrate content of those commercial formulas compared with the composition of the present invention (Formula A). Natural bitch milk was used as a baseline value (100)*.

TABLE 1

	Bitch	ESB	JBD	Nurtal	Formula A
Protein	100	83.1	74.4	78.8	96.6
Fat	100	132.6	107	103.6	95
Carbohydrates	100	69.3	114.4	115.3	103.9

As can be seen from Table 1, and as shown in Fig. 1 which is a bar graph of the data shown above, the composition of Formula A is much closer to natural bitch milk than the other products.

The fatty acid and amino acid profiles of the commercially available products were also analyzed and compared with bitch milk and the composition of the present invention as shown below in Tables 2 and 3. The comparisons are expressed as a percent of control (bitch=100%). The values for the amino acids and fatty acids are expressed as a percent of total protein and total lipids, respectively.

*The milk fat values were obtained from Lonnerdal et al., "Developmental Changes in the Composition of Beagle Dog Milk", *Am. J. Vet. Res.*, Vol. 42:662-666 (1981) and Oftedal, "Lactation in the Dog: Milk Composition and Intake by Puppies", *J. Nutr.*, 114:803-812 (1984).

TABLE 2 - FATTY ACID PROFILE

	Control	ESB	JBD	Nurtal	Formula A
Palmitic	100	44.4	58.4	53.9	70.8
Stearic	100	517.2	90.3	97.6	251.7
Oleic	100	78.1	73.4	71.8	97.7
Linoleic	100	77.2	309.7	337.9	133.1
Arachidonic	100	6.5	0	0	74.6
DHA	100	8.6	0	0	42.9
Trans Fatty Acids	100	577.9	17.6	0	62.9

TABLE 3 - AMINO ACID PROFILE

	Bitch	ESB	JBD	Nurtal	Formula A
Arginine	100	84.6	71.5	95.4	79.3
Histidine	100	54	55.7	73.3	99.1
Isoleucine	100	106.7	99.8	107.7	108.1
Leucine	100	77.2	68	79.3	82
Lysine	100	92.1	146.6	145.9	140.9
Methionine	100	230.3	47	71.7	79
Cystine	100	63.8	41.5	53.6	111.6
Phenylalanine	100	105.4	112.3	135.2	118.5
Tryrosine	100	110.6	290.7	132.5	90.4
Threonine	100	120.3	78.8	93.2	123.7
Tryptophan	100	124.6	64.9	163.2	168.4
Valine	100	85.9	78.6	91.1	89.8

Figs. 2 and 3 are bar graphs illustrating the data from Tables 2 and 3, which clearly show that the fatty acid and amino acid profiles of the composition of the present invention is much more similar to the bitch milk profile than any of the other products.

Example 2

To study the effect of diet on serum fatty acid profile and amino acid profile, forty colony bred Beagle puppies from 7 litters were assigned to three treatments: bitch milk (CTL), (n=15) milk replacer I (MR-I) (n=14) comprising 40% protein and 30% fat (an example of the present invention), and milk replacer II (MR-II) (n=11) comprising 33% protein and 40% fat (Esbilac® Milk Replacer for Puppies from Pet-Ag, Inc.). All puppies were allowed to nurse the dam for 24 hours with subsequent milk replacer feedings every 3 hours for days 1-2 and gradually increased to every 6 hours over the duration of the study. MR-I was found to contain higher levels of essential (EAA) and non-essential (NAA) amino acids than MR-II. Puppies fed MR-I were more similar to CTL in serum EAA and EAA:NAA ratio. These data indicate that the serum fatty acid profile and amino acid profile of growing puppies can be influenced by and are reflective of the milk replacer formulation.

Example 3

To the determine the effect of milk composition on growth and body composition of puppies, forty colony bred Beagle puppies from 7 litters were randomly assigned to three treatments: bitch milk (CTL) (n=15), milk replacer I (MR-I from Example 2) (n=14), and milk replacer II (MR-II from Example 2) (n-11). All puppies were allowed to nurse the dam for 24 hours. Milk replacer treatments were subsequently fed to the puppies every 3 hours and decreased to 4 feedings/day for the remainder of the study (30 days). No differences were detected in intake between the MR puppies; however, puppies fed MR-I had increased average daily weight gain and gain efficiency over MR-II fed puppies, i.e., when the amount of product consumed was measured against the weight gained, MR-I fed puppies had a greater increase in body weight, indicating that the MR-I formulation is better at meeting the growth needs of puppies. The body composition of puppies fed MR-I did not differ from CTL puppies in body fat

percentage, but was higher in lean tissue than both CTL and MR-II. Puppies fed MR-II were found to have the highest body fat and lowest lean tissue. These data indicate that the MR-I formulation, which was more similar to bitch milk in fatty acid profile and amino acid profile, results in enhanced structural tissue growth indicating an improved nutritional status in neonatal puppies.

While certain representative embodiments and details have been shown for purposes of illustrating the invention, it will be apparent to those skilled in the art that various changes in the methods and apparatus disclosed herein may be made without departing from the scope of the invention, which is defined in the appended claims.

What is claimed is: